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TREATMENT METHODS IN EXPERIMENTAL ENDOTOXIN SHOCK

by

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The undersigned certify that they have read and recommend to the Faculty of Medicine for acceptance a thesis entitled "Treatment Methods in Experimental Endotoxin Shock" submitted by Lawrence Joseph Clein in partial fulfilment of the requirements for the degree of Bachelor of Science. (Medicine).

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ABSTRACT

The syndrome of bacteremic or septic shock, represented by the triad of a septic focus, hyperpyrexia and hypotension is being recognized with increasing frequency. As a complication in surgical patients it produces a high morbidity and mortality.

In analysing 53 Gram-negative blood cultures from all the services of the University of Alberta Hospital over a three year period, a total of 17 patients died, and in 11 this was due to septic shock. Another 14 patients developed shock but recovered. During the six year period 1956-61, 41 patients died from septic shock on the surgical services of the University of Alberta Hospital.

This significant mortality figure has prompted us to investigate resuscitative measures in the experimental animal. The endotoxin shocked dog simulates many of the features of septic shock in humans, and for this reason this preparation has been used as a convenient experimental model. Shock was produced in 106 mongrel dogs using lipopolysaccaride E. coli O111:B4. A further 10 dogs were just cooled acting as controls for the hypothermia experiments.

Thirty dogs received treatment prior to the administration of endotoxin; 54 dogs after shock had been produced. Twenty-one dogs received endotoxin and no treatment and served as controls. Endotoxin was given intravenously in a dose of 5 mgm/kgm. This dose was previously found to be uniformly lethal to all dogs in 12 to 24 hours. Pre-shock treatment included 1) phenoxybenzamine hydrochloride (Dibenzyline), 2) heparin, 3) hypothermia. Post-shock treatment included 1) extracorporeal circulation, 2) extracorporeal circulation plus Dibenzyline, 3) extracorporeal circulation plus Dibenzyline in previously cooled animals, 4) hypothermia, 5) Dibenzyline.

Following the injection of endotoxin in the dog the blood pressure drops precipitously in the first few minutes quickly rising again to almost the original level. This is followed by a slower second fall accompanied by vomiting, tenesmus, rectal bleeding and progressive deterioration to death in 12 to 24 hours. Hyperpyrexia is a common occurence in the terminal stages, though hypopyrexia was occasionally seen. All control animals died in an average time of 15.9 hours. In the groups in which extracorporeal circulation was used, its use alone did not significantly increase survival time. However, when Dibenzyline was infused intravenously over the same hour as partial bypass, there were five survivors with the remaining animals living significantly longer than the controls. When this last technique was employed in previously cooled animals the survival time was reduced below that of the controls.

All dogs cooled as described and allowed to rewarm without any further insult survived. When the dogs were cooled prior to the injection of endotoxin, one survived, while the average survival time of 21 hours in the remainder represents a significant increase over normal. However, cooling one hour after the initiation of endotoxin shock hastened the demise of the animals.

Dibenzyline, a potent adrenergic blocking agent, has been the most interesting resuscitative agent in our hands. When given to 8 dogs prior to endotoxin it resulted in five survivors, a highly significant survival rate. Although no dogs survived post-treatment with Dibenzyline the average length of survival was significantly increased. The use of Dibenzyline in combination with partial bypass has already been mentioned. Dibenzyline is the common denominator in all animals showing a significant increase in survival time, and we have been sufficiently impressed with it to use it on selected patients.

The efficacy of Dibenzyline in four moribund patients is described in the addendum. All patients had previously received vasopressors, steroids, and blood transfusions and when seen were still hypotensive, oliguric or anuric and gravely ill. In one patient who developed intestinal obstruction following an ileal conduit procedure, Dibenzyline had no effect. The other three patients recovered from a severe state of shock following the intravenous infusion of Dibenzyline.

CHAPTER I.

The problem of shock appears to be as old as medical history itself. In all the great eras of medicine, descriptions of the syndrome of shock are to be found in one form or another. There is little doubt that the patient Hippocrates observed, whose facial appearance has been recorded for all posterity, was in shock. Le Dran in 1743 first coined the word "choc" though he used it to designate the act of collision rather than the resulting functional damage (54). Over the next one hundred years or so the word gradually came to have the connotation by which it is understood today.

Any thesis relating to the problem of shock would be incomplete without an attempt at definition. However, there is no denying that a problem of definition exists. Cannon wrote in 1923 "It seems to me that, in such a complex as shock, definition is not a prime requisite. The important matter is to obtain a careful description of the observed facts." (10). Shock is a complicated clinical syndrome characterized by hypotension, tachycardia, pallor, and various degrees of disturbance of consciousness in which one feature may predominate over others, and which results from a variety of pathological conditions. As a result of one of these pathological conditions whether it be trauma, burns, infection, blood loss, there arises a discrepancy between the capacity of the vascular bed and the volume of blood available to fill it. There is progressive reduction of the nutritive blood flow through the parenchymal tissues to the point where the capillary circulation deteriorates and venous return to the heart is no longer effectively maintained. Tissue anoxia results and the irreversibility of shock derives directly from this factor. (29, 31, 37, 39, 5

All forms of shock proceed through two main phases - an initial stage of compensation in which the peripheral circulation is sustained by homeostatic readjustments in the tissues and by the activation of specialised centres (34); the compensatory status is maintained for a variable period until separate aspects of the compensatory response begin to deteriorate, after which the second decompensatory

stage sets in. Recent research in shock has been concerned with attempts to identify the mechanisms precipitating the second phase of the circulatory collapse. One of the mechanisms which has been suggested is the systemic dissemination of bacterial endotoxin. (15, 16, 17, 19).

The tendency to decompensation during the terminal phase of shock in large part results from alterations in the intestinal tract and liver (20, 27, 28, 30). Two separate explanations for the "toxic" factor in shock have been advanced, each placing major emphasis on changes in one of these structures. Shorr believed that the key factor responsible for the decompensatory aspect of irreversible shock is a progressive depression of vascular reactivity mediated by an uncontrolled release of iron. (49). This, apparently, is a consequence of stagnant hypoxia in the liver and its associated impairment of ferritin metabolism. On the other hand, Fine and his co-workers emphasised the effect of reduced blood flow through the intestinal tract in shock, leading to a systemic dissemination of bacterial products, such as gram-negative endotoxins. (19,20,21,23,48).

Endotoxin is the term commonly used to designate a complex lipopolysaccharide which is produced by Gram-negative bacteria. It has antigenic specificity by virtue of its polysaccharide component. Its toxic properties are not necessarily neutralized by antibody and are generally similar regardless of the specific source of the endotoxin. (4,7,29,46, 51).

It is a high molecular weight phospholipid polysaccharide protein complex. The lipid fraction is important for toxicity (46). Endotoxin has several synonyms including bacterial pyrogen, somatic antigen, Boivin antigen, tumour necrotising substance and Shwartzman toxin.

In 1956 Thomas showed that when epinephrine or norepinephrine was injected into the skin of rabbits shortly after an intravenous injection of endotoxin, hemorrhagic necrosis of that area of skin occurred. Similar lesions were produced by the intradermal injection of epinephrine and endotoxin together. Pretreatment

with cortisone, phenoxybenzamine hydrochloride and chlorpromazine, prevented the lesions from occurring (52). Zweifach and co-workers studied the effect of epinephrine and endotoxin in greater detail in the rat mesoappendix and isolated rabbit ear preparation (55, 56, 57). In both instances sublethal doses of endotoxin resulted in greatly augmented and prolonged vasoconstrictor responses to epinephrine and norepinephrine in terminal arterioles and venules. Larger lethal doses of endotoxin resulted in vasodilation with pooling of blood in the small vessels while the larger arterioles and venules exhibited increased vasoconstriction. More recently it has been suggested that endotoxin mediates this altered reactivity to epinephrine through the release of Serotonin (47).

The possibility that an endotoxaemia is the cause of irreversibility of shock of all types has occurred to several workers in recent years (18, 19, 20, 21, 23, 33, 37, 45). Cannon first postulated the theory of a toxic factor over three decades ago (11). Aub in 1944 was the first to suggest a bacterial toxin. Following the death of dogs in irreversible tourniquet shock, he was able to isolate clostridial organisms from the damaged muscle. (5). That clostridial exotoxin is not the cause of irreversible shock is now fairly well established for the following reasons: (1) the morbid anatomy of irreversible hemorrhagic and septic shock is unlike that produced by these exotoxins, (2) clostridial antitoxin has no curative action, (3) penicillin is not as helpful as it should be in clostridial infections, and (4) these bacteria cannot be implicated in other animals, in the tissues of which clostridia are rarely found. In 1951, Shorr and coworkers (49) were in favour of a toxic factor because they found that a saline extract of shocked liver yeilded a substance, VDM, later identified as a reduced ferritin which was found to depress the sensitivity of the rats' mesoappendix to topically applied epinephrine. Zweifach (57) has sugsequently provided further data invalidating the VDM hypothesis.

During the last twenty years Fine and his workers have accumulated a considerable amount of evidence to support their contention that endotoxin is the cause of irreversibility of all forms of shock. In this they include shock brought on by sepsis, hemorrhage and trauma. Their evidence is briefly reviewed below.

If a toxin is present it must be shown to be capable of killing not only a test animal, but the donor animal from which it was obtained, and by the same mechanism in both. In order to investigate the toxin in a recipient animal, that animal has to have a weak detoxyfying power in order to be able to reveal a small amount of toxin. Such an animal is a rabbit or a dog exposed to two hours of hemorrhagic shock, which will survive if transfused with its own blood at that time, but which dies if exposed to the toxic factor from the donor dog. The experiment was performed as follows: liver mash from a dog dying of shock, was injected intraperitoneally into several normal dogs. The dogs survived with almost no untoward signs. The same was done with recipient dogs described above with dramatically different results in that the potentially reversible shocked dog reverted to a severe state of shock exhibiting all the manifestations of irreversibility. This was strongly suggestive of a toxin in the shocked liver. That it was of bacterial origin was observed when the recipient was prevented from going into irreversible shock by pretreatment of the donor with antibiotic. The next step was to identify the toxin in the blood. When the donor dogs' blood was transfused into normal dogs, again nothing untoward occurred, but when it was transfused into the suitably prepared recipients, they died of irreversible shock. Again protection was obtained for the recipients by pretreating the donor with antibiotics. The same tests have been repeated in animals subjected to tourniquet and septic shock.

Fine has more recently isolated a lipopolysaccharide fraction from the plasma of shocked animals which is not present in the plasma of normal animals.

This has been shown to have the biological properties of bacterial endotoxins, such as pyrogenicity, the ability to produce the generalized Shwartzman reaction and to induce resistance to a lethal dose of endotoxin. Fine has also shown that germ free animals are protected against lethal shock in some cases, or the fatal outcome is greatly delayed (21). Hardy and co-workers have failed to prevent shock by pretreatment with antibiotics (33). Fine suggests that this was due to the fact that their animals had a resistant strain of organisms present. Zweifach et al. were unable to show any difference in lethal outcome in germ free rats (58). Einheber quoting Carl Wiggers says, "While it is easy to postulate the existence of toxic agents, it has proved far more difficult to demonstrate their presence." This worker has repeated many of Fine's experiments, but has been unable to obtain similar results (18).

These experiments of Fine's, in particular the isolation of an endotoxin-like substance from the blood of animals in shock, strongly supports the hypothesis that endotoxin acts to perpetuate the hypotension of shock, whatever its initiating cause may have been. Against this hypothesis are the facts that, 1) germ free animals have not been shown to be more resistant to hemorrhagic shock and 2) not all workers can confirm the observation that antibiotics protect against hemorrhagic shock.

The triad of a focus of infection, hyperpyrexia and hypotension comprises the syndrome of septic shock. A review of the literature suggests that this syndrome is appearing with increasing frequency (2,3,9,12,13,31,39,45,50). While septic shock may occur with almost every infectious process, certain clinical pictures have been recognised as being more frequently associated with the development of shock. These include 1. infection following genito-urinary instrumentation, 2. peritonitis and intra-abdominal abscesses, 3. biliary

disease and surgery, 4. leukaemia, lymphoma and carcinoma, and 5. infections of the parous female genital system. The commonest organisms found either in the blood stream or infected lesions of patients in septic shock are the Gram-negative coliforms which are the same organism most commonly associated with the production of endotoxins. Thus endotoxin shock has become synonymous to a certain extent with Gram-negative bacteremic shock. That endotoxin shock is synonymous with all types of septic shock is by no means widely accepted.

The clinical picture of septic shock is fairly characteristic. However at times, particularly when it occurs shortly after surgery, septic shock may be confused with hemorrhagic shock. An abrupt rise in temperature often accompanied by a rigor usually precedes the onset of shock. A rigor occurring in the post-operative period is an ominous sign and should alert one to watch particularly for the onset of shock. A blood culture examination is warranted and blood should be withdrawn at the height of each and every rigor, as often as possible, before antibiotic therapy is commenced. Hypotension is the most characteristic finding and usually follows the sudden rise in temperature within twelve hours. In one series of patients (12) the average fall in blood pressure was 58 mm. Hg. Hall and Gold (31) described two clinical pictures. In one group of patients the skin of the extremities was pale, cold and moist; the pulse was weak and thready. The sensorium was clouded and the patients exhibited apathy and listlessness. In another group the skin was warm and dry and the pulse full and bounding. These patients were bright, alert and active.

To summarise this chapter, the pathophysiology of shock appears to be closely linked with bacterial endotoxins. Although the evidence that an endotoxemia as the basis of irreversible shock is impressive, it is still largely circumstantial. However, endotoxins are considered to be the cause of the majority of clinical

bacteremic shock by most authorities (2, 3, 9, 12, 13, 31, 39, 45, 50).

Endotoxin shock is appearing with increasing frequency in the wards of hospitals and the mortality rate is high. This would suggest that the present day therapeutic regimes are inadequate.

This work is to report the results of new methods of therapy in the experimental animal with some additional results in clinical cases.

CHAPTER II.

Materials and Methods.

A total of one hundred and sixteen mongrel dogs of both sexes weighing between eight and twenty kilograms comprise the animal material of this study. All dogs were maintained in the kennels for a minimum of one week prior to the experiment and were pronounced free from disease by the veterinary surgeon. During this period the dogs received antidistemper vaccine and standard kennel diet. On the day prior to surgery food was withheld, but water was allowed ad libitum.

The endotoxin used in this study was lipopolysaccharide E. coli O111:B4. In the desiccated state this lipopolysaccharide is stable at room and refrigeration temperature. The rehydrated solution is quite stable when refrigerated to prevent bacterial contamination. There is a slow deterioration of potency in the liquid state but this occurs over about three months. Thirty dogs received treatment prior to the administration of endotoxin; 54 dogs after shock had been produced. Twenty-one dogs received endotoxin and no treatment acting as controls. Endotoxin was given intravenously in a dose of 5 mgm/kg. This dose was previously found to be uniformly lethal to all dogs in 12 to 24 hours. Unlike testing the effects of a drug upon an experimental animal when an MLD_{50} dose of drug would be most likely to reveal the minimal therapeutic effects of associated treatment, we are here testing the effects of a method of treatment. It was thought, therefore, that in order to give the treatment method the most stringent test, an MLD_{100} dose of endotoxin was warranted.

Preshock treatment included 1) phenoxybenzamine hydrochloride (Dibenzyline), 2) heparin, 3) hypothermia. Postshock treatment included 1) extracorporeal circulation, 2) extracorporeal circulation plus Dibenzyline, 3) extracorporeal circulation plus Dibenzyline in previously cooled animals. 4) hypothermia 5) Dibenzyline (Table I). All dogs were anesthetized with sodium pentobarbitol thirty milligrams per kilogram body weight. The dogs were placed upon the operating

table in a supine position, and in all animals the femoral artery and vein of one side were cannulated. The blood pressure was monitored throughout by connecting the femoral artery cannula to a mercury manometer.

Endotoxin was administered as an aqueous solution of 20 miligrams per cc. Within a minute the blood pressure drops precipitously and then rises again in another three or four minutes to almost the original level. About an hour later it starts to fall again more slowly, but progressively until death occurs some ten to twenty hours later. In the anesthetized dog there are few other symptoms or signs, though the passage of a loose, bloody stool is quite common. In the unanesthetised dog the clinical picture is quite characteristic. Following the injection of endotoxin, there is usually a forced bowel movement. This is followed in sequence by tenesmus, retching and vomiting, bloody diarrhoea, total lack of interest in food and water, progressive ataxia, coma and finally death. Hyperpyrexia is usually present, though the temperature may fall below normal. At autopsy gross findings include an enlarged congested liver, hemorrhagic necrosis of the small and large bowel, petechial hemorrhages in the lungs and myocardium. The kidneys and adrenals are quite normal to gross appearance.

Phenoxybenzamine hydrochloride (Dibenzyline) was used as an aqueous preparation in a dose of three milligrams per kilogram body weight. This was diluted in 250 ccs. of glucose and saline and was considered more than an adequate dose in achieving the required adrenergic blocking effect. Dibenzyline was given intravenously over a period of one hour in all groups in which it was used, that is, by itself in a pretreatment group, and in posttreatment groups, by itself, and in combination with extracorporeal circulation, and extracorporeal circulation and hypothermia. (Table I).

TABLE I

METHODS OF RESUSCITATION

116 DOGS

	Number of Dogs
I - Pre-Shock	
A. Dibenzyline	8
B. Heparin	7
C. Hypothermia	15
II - Post-Shock	
A. Extracorporeal circulation	9
B. Extracorporeal circulation Dibenzyline	19
C. Extracorporeal circulation Dibenzyline with precooling	6
D. Hypothermia	15
E. Dibenzyline	6
III - Controls	
A. No treatment	21
B. Hypothermia controls	10

Heparin 1:1,000 was given intravenously in a dose of six milligrams per kilogram body weight in the one pretreatment group. Heparin was also given to all groups receiving extracorporeal circulation in a dose of two milligrams per kilogram. It was not given to any of the hypothermia groups. (Table I).

Hypothermia was carried out in the control, pretreatment and posttreatment groups alike, by covering the anesthetised dog with ice chips placed in polythene bags or placed loosely over the chest and abdomen. By this means the temperature could be reduced from a mean of 37.5°C to 30°C. in approximately one hour. When the temperature, which was measured rectally, reached 30°C, the ice was removed, and the dogs allowed to rewarm. Before this occurred the temperature usually fell to about 28°C. before starting to rise. In the control groups this drift took about three hours, while another six to eight hours was required for the dog to reach normothermic levels again. Shivering was usually prevented by an injection of a further $\frac{1}{2}$ cc. of Sodium Pentobarbital. (Table I).

Partial extracorporeal circulation was performed by cannulating a jugular and femoral vein for the venous component of the system. These cannulae were placed in the superior and inferior venae cavae respectively. A femoral artery was cannulated and the cannula passed up into the aorta, for retrograde arterial perfusion. The pumping unit was composed of two model T - 6S Sigmamotor pumps. The pumping action resulted when the cam-operated fingers pressed in sequence against flexible tubing. A positive unidirectional movement was imparted to the fluid contained in the tubes. The top of the pump was hinged to allow the tubing to be inserted after sterilization, without contamination. No contamination of the fluid in the tubing occurred from the pumps. Back flow was prevented by a continuous closure of the tubing by at least one finger. (Table I).

The oxygenator was of the disposable plastic bag bubble type (pulmo-Pak) for use in association with the pump just described.

Preliminary trials with the use of partial bypass revealed that if it was prolonged for a sufficient length of time, all the animals died. This critical time was approximately four hours. The animals appeared to die in a shock-like state. However, similar preliminary trials revealed that there were no untoward side-effects or deaths when partial bypass was maintained for only one hour. This was the reason for choosing this time. The limitations of the pump-oxygenator system used in these experiments are well recognised. The main problem is the destruction of red blood cells which occurs with a sigmamotor pump after a relatively short time. This difficulty is mentioned to emphasise that the beneficial effects of assisted circulation plus Dibenzylene mentioned below may have been greater still had a less traumatic pump-oxygenator system been available.

Pretreatment Groups.

In these groups treatment methods were carried out before the induction of endotoxin shock, in other words these groups of animals were prophylactically treated. Three groups are included here. Eight dogs received Dibenzyline (3 mgm/kg), twenty four hours prior to shock. Seven dogs received Heparin (6 mgm/kg), half an hour before shock. Fifteen dogs were cooled to a temperature of 30°C, at which point endotoxin was administered.

Posttreatment Groups.

One hour after the injection of endotoxin was chosen as an arbitrary time to commence therapy in the posttreatment groups. At this point six dogs received intravenous Dibenzyline, slowly infused over one hour; fifteen dogs were cooled to 30°C, and then allowed to rewarm; and a total of thirty-three dogs received partial extracorporeal circulation. Of these, nine received it alone for one hour; eighteen received it in combination with Dibenzyline infusion for one hour;

and six received it in combination with Dibenzyline for one hour, having been previously cooled to 30°C before endotoxin was given. This last combination was chosen, as it was felt from early experiments that precooling, and treatment with extracorporeal circulation and Dibenzyline were producing the best results, and that this combination might produce the maximum number of survivors. As can be seen from the results this did not occur.

Results.

The results are summarised in Tables II, III and IV. In the pretreatment groups, five out of eight dogs receiving Dibenzyline survived, but the survival time of the remaining three was not significantly increased over controls. (Table II). The survival of five dogs in this group is highly significant. While one dog survived, fourteen dogs that were cooled prior to receiving endotoxin lived an average time of twenty one hours. This is a significant increase in survival time ($p < .05$). (Table III). The dogs pretreated with Heparin did not live as long as the controls.

In the groups treated after the induction of endotoxin shock, the group of particular interest is that treated by extracorporeal circulation and Dibenzyline. (Table IV.) In this group five out of eighteen dogs were full time survivors while the remaining thirteen dogs lived significantly longer before dying ($p < .01$). The only other group that lived significantly longer than controls was that treated by Dibenzyline which lived an average twenty-four and a half hours ($p < .05$). None of the other groups had significantly increased survival times.

The statistical methods employed in calculating the results are:

1). The standard error of the difference for the survival time in hours.

2). The chi-squared test for the survivors.

TABLE II

DIBENZYLINE

Groups	No. of Dogs	No. of Survivors	Average Survival Time in Hours
Controls No treatment	21	0	15.9
Dibenzyline Pre-treatment	8	5x	19.3*
Dibenzyline Post-treatment	6	0	24.5 †
Extracorporeal circulation plus Dibenzyline	19	5 +	35.7*

* $p < .01$ † $p < .05 > .01$ + $p = .05$

x $p = .001$

TABLE III

HYPOTHERMIA

Groups	No. of Dogs	No. of Survivors	Average Survival Time in Hours
Controls No treatment	21	0	15.9
Hypothermic controls	10	10	-
Hypothermic Pre-treatment	15	1	21.0 †
Hypothermia Post-treatment	15	0	12.33
Extracorporeal circulation plus Dibenzyline with precooling	6	0	12.6

† $p < .05 > .01$

TABLE IV

EXTRACORPOREAL CIRCULATION

Group	No. of Dogs	No. of Survivors	Average Survival Time in Hours
Controls No treatment	21	0	15.9
Extracorporeal circulation Alone	9	0	20.8
Extracorporeal circulation plus Dibenzyline	19	5†	35.07*
Extracorporeal circulation plus Dibenzyline with precooling	6	0	12.33

* $p < .01$ † $p = .05$

CHAPTER III

The considerable success in the treatment of hemorrhagic shock has focussed the interest of physicians in medical, surgical and obstetrical specialties on the less successfully treated shock associated with bacteremia. Although shock associated with sepsis has been known to clinicians since at least 1831,(54) the mechanisms of its production have received little attention until recently. The commonest type of septic shock is that found in association with infection due to Gram-negative organisms, and it has been related to the release of endotoxin.

The concept has been developed that all types of shock may ultimately become irreversible as a result of a bacterial factor, such as endotoxin. It is realised that this is not universally accepted. However it is generally agreed that bacteremic shock and endotoxin shock are synonymous, particularly when the organisms responsible for the former are of the Gram-negative variety. Whether irreversibility be due to endotoxemia, coagulation defects, the release of histamine or serotonin or intestinal lesions, all workers are in agreement that a complex of situations exists which culminates in circulatory collapse. It has become apparent that variations among species, and even within species, make generalizations concerning the shock syndrome a misleading oversimplification (59). Among the variables which influence the progression and severity of shock in experimental animals are factors such as age, sex, season, nutritional state and intercurrent infection. This last factor is of particular importance if a bacterial toxin is incriminated. As a corollary to this, intercurrent bacteremia renders animals more susceptible to shock. Such infections are encountered consistently in rabbits, where, for example coccidiosis is an inevitable contaminant. Rats are invariably infected with pleuropneumonia organisms, while most monkeys used for research today have

tuberculosis (59).

Bearing this in mind, it is conceivable, though probably unlikely that experimental shock is a different entity from clinical shock.

Another question which cannot be overlooked, but which is just as difficult to overcome, for the obvious humanitarian reason, is that of anesthesia. This factor inevitably distorts the vascular responses in the shocked animal (20, 23). It is virtually a sine qua non never to anesthetise a shocked patient. In surgical patients, which constitute the majority of day-to-day shock cases, every attempt is made to eliminate many of the factors which may be responsible for the death of experimental animals. Bowel infection is minimised since the patient is treated with antibiotics, the intestinal tract is sterilised and fluid balance is carefully maintained. Hypoxia, characteristic of animal shock, is never allowed to occur. Suitable medication and blood replacement is always at hand. Shock in patients, in other words, usually develops in spite of intensive therapy, which is increasingly intensified, once the condition manifests itself.

These features are mentioned to emphasise the difficulty which obtains in attempting to extrapolate animal experiments to human disease conditions.

What is the rationale for attempting to pre-treat animals? Actually two reasons exist. First by pre-treating an animal it may be possible to elucidate some of the mechanisms whereby shock develops. As has been mentioned, experimental shock may be a different entity, or at any rate a vastly different stage of development from human shock. It is obviously illogical to attempt to pre-treat human patients. Prophylactic therapy is always carried out, and the development of shock prevented, whenever possible. The second reason therefore, for pretreating animals is that clinical shock as is seen in the early stages

following trauma or surgery may be a very early manifestation of experimental shock, and pretreatment, which in animals prevents the onset of irreversibility, may well be a life-saving measure in patients.

Pretreatment with phenoxybenzamine hydrochloride (Dibenzyline) has been known for some years to prevent a lethal outcome in animals subjected to hemorrhagic, traumatic or endotoxin shock (6, 37, 41, 42, 53, 54). This has been confirmed by us with five survivors out of eight animals so treated. Dibenzyline is a potent adrenergic blocking agent of the beta-haloalkylamine series. Physically Dibenzyline occurs in the form of colourless crystals. It is insoluble in water, but soluble in warm propylene glycol. It is unstable in neutral and basic solutions, so that an aqueous suspension or an aqueous propylene glycol or alcohol solution of the preparation must be stabilised with added acid to prevent slow hydrolysis.

Following the insult (hemorrhage, trauma, burns, endotoxin) used to provoke a state of shock, a widespread compensatory vasoconstriction occurs (51, 52, 55, 56, 57). If this vasoconstriction is prolonged it becomes ultimately harmful, and in fact the anoxia which results from reduction in blood supply to the tissues subsequently gives rise to the decompensatory vasodilatation which heralds irreversibility. If the compensatory vasoconstriction therefore is ultimately responsible for irreversibility, it has been suggested by Nickerson and co-workers (41, 42) that a vasodilator drug might be more useful at this stage than trying to further vasoconstrict with the vasopressor drugs currently being used in the treatment of hypotension. This has been the rationale for the use of Dibenzyline in the experiments described. As has already been mentioned pretreatment has a protective action against endotoxin shock, but the combination of Dibenzyline and extracorporeal circulation has produced survivors in animals that had been in severe shock at the time therapy was commenced. Dibenzyline by itself, once shock was established, did

not result in any survivors.

One of the most recent theories to be put forward is that sludged blood combined with resulting intravascular agglutination, thrombosis and fibrinolysis is the basic factor responsible for the development of irreversible shock. Changes in the coagulation system have been shown to occur by Gans and Krivit (26,27,28) and Hardaway and co-workers (32) as evidenced by:-

1. Decrease (due to using up) of blood clotting elements including fibrinogen.
2. Finding of thrombi in tissue sections.
3. Finding of focal necrosis and infarcts.
4. Prevention of these findings by preheparinization.

It has been postulated that the irreversible state results from a Shwartzman-type of reaction following absorption of endogenous endotoxin from the intestine previously damaged by exogenous endotoxin. A prime characteristic of such a reaction is that it can be prevented by pretreatment with heparin in the experimental animal (30). Gans and Krivit and Hardaway et al report success with this measure. Using an MLD_{50} dose of endotoxin all animals pretreated with six milligrams of heparin/kg. survived. However, using an MLD_{100} dose of endotoxin all animals succumbed in spite of heparin pretreatment. Lillehei, Longerbeam and Rosenberg (38) using thirty milligrams of heparin/kg. were unable to produce survivors, and concluded that as heparin did not alter the outcome of the irreversible state in any way, it indicated that widespread small vessel thrombosis as a result of Shwartzman-type reaction was apparently not a factor in the etiology of the irreversible state. As shown by the experimental results obtained by us, we must concur with the last statement. It is interesting to note that Matthes (40) has treated a patient in septic shock, with heparin, noradrenaline and antibiotics, who recovered completely. Two other patients treated with heparin, however, died.

Allen, Estes and Mansberger (1) reported three patients in septic shock treated by hypothermia with good results. Hypothermia was utilized only after

the failure of the patients to respond to the accepted therapeutic regimes. They also report an interesting case illustrating the prophylactic use of hypothermia in a patient in whom bacteremic shock was a decided possibility. Cockett and Goodwin (14) describe fifteen urological patients in whom septic shock developed. All had undergone a surgical procedure. Eleven of twelve patients treated by hypothermia recovered. Three patients treated by conventional methods died. Overton and Debakey (43) utilizing hypothermia in hemorrhagic shock showed that precooling prolonged survival time of dogs, but did not prevent death. Friedman, Davidoff and Fine obtained similar results and also showed that the protective effect of cooling was not obtained if applied after the induction of hemorrhagic shock (25). According to Blair and coworkers (8) who employed hypothermia in the treatment of thirty-three patients with septic shock with seventeen survivors, "of special importance is the cold pressor effect which elevates and sustains the arterial blood pressure." In the animal experiments hypothermia consistently lowered the blood pressure even in the control animals that did not receive endotoxin. It is apparent from the experimental results that hypothermia is not a recommended procedure in the attempted resuscitation of the shocked dog. It is difficult to explain the beneficial effects obtained clinically, although the rationale for the use of hypothermia is well founded. If irreversibility of shock is due to anoxia and the release of toxic products, hypothermia, by reducing the oxygen requirements of the tissues, is a logical choice particularly as a prophylaxis. However, if the irreversible state has already been reached, whatever the toxic products of anoxia may be, they are already in the circulation and hypothermia is unlikely to get them out. This fact supports the theory that there may be a considerable degree of difference between experimental and human shock. Experimental endotoxin shock in dogs is potentially irreversible the instant that the endotoxin is released into the blood stream and the remarkable thing is that these animals take so long to die. Shock in humans probably

becomes irreversible only terminally and irreversibility precedes death by a few minutes. This would explain the apparent discrepancy in results obtained in the therapeusis of animals and humans by hypothermia. Hypothermia, therefore, can be employed prophylactically with advantage, but as a therapeutic measure it should be used with extreme caution in patients, as there is really no good experimental evidence to support its use.

The concept of maintaining the circulation by artificial extracorporeal means has intrigued man for many years. However, it is only comparatively recently that blood pumps and artificial oxygenators have been designed which are capable of supporting the circulation of an entire animal. This technical development has realized the most outstanding advance in surgery in recent times, namely intracardiac operations under direct vision.

When blood drawn from the venous system is aerated and pumped into the arterial tree, two possible situations exist. The first known as "partial perfusion," is that in which a portion of the venous return continues to pass through the heart and lungs. The second is encountered during "total perfusion" in which the venae cavae are entirely occluded to prevent blood from entering the right atrium. The term "cardiopulmonary bypass" has been coined and is frequently used in surgical literature. In the experiments described in the previous chapter partial perfusion was employed. The limitations of the pump oxygenator system used have been discussed in that section. Cardiopulmonary bypass certainly allows for a better perfusion because of the increased venous return and thus better flow rates obtained. This is particularly so in a small animal such as the dog. But partial perfusion was utilized for the obvious reason that if an extracorporeal technique was to be employed in the resuscitation of a shocked individual, then a thoracotomy would be definitely contra-indicated, and the simpler the surgery necessary (in this case the cannulation of an artery and vein), the better it would be.

Shock has been defined as a discrepancy between the capacity of the vascular tree and the blood available to fill it. As a result of this discrepancy there is a decreased venous return to the heart, the cardiac output falls, the blood pressure drops, and the vicious cycle has commenced. The rationale behind the use of extracorporeal techniques is to supplement the low cardiac output and thus maintain perfusion of organs and tissues in an attempt to reduce the lethal anoxia which otherwise occurs.

Much information has been amassed by the perfusion of isolated organs. Thus Lillehei and MacLean (35, 36) perfused the intestine through the superior mesenteric artery in dogs in hemorrhagic and endotoxin shock. When the flow of blood to the gut was maintained and these tissues protected from hypoxia, irreversible shock did not occur. Delorme (16) perfused the liver in dogs, again preventing irreversible shock when the liver was well oxygenated. Cerebral cross-circulation perfusion was carried out by Penner and Klein (44) who showed that the central nervous system was primarily involved in the development of irreversibility. However, no one has been able to repeat this latter work.

All isolated organ perfusions are technically impossible because of the surgery involved, in treating patients in shock. It was reasoned that if isolated organ perfusion was so beneficial, then total body perfusion should be equally beneficial. Cowely and colleagues have employed whole body partial perfusion in dogs in hemorrhagic shock (15). In twenty animals perfused for one hour there was an increase in survival time. There were no survivors, however, and gross autopsy appearances showed no difference between perfused animals and controls. Dickson, Hamer and Dow (17) have developed a closed system for veno-arterial pumping without oxygenation. The system is stable and non-traumatic and apparently suitable for prolonged use. One dog survived fifty-two hours with no disturbance in blood, renal function or acid-base balance. They suggest

the system would be useful for patients with cardiac infarction, congestive heart failure and shock. However, the oxygen saturation falls progressively at a flow rate similar to that used by us to an average of 62 per cent. This added hypoxia, to that already present by virtue of the shock, would hasten the demise of a shocked animal. For this reason an oxygenator is essential and consequently perfusion can only be maintained for a limited time.

As can be seen in the results tabulated in the previous chapter extracorporeal circulation and Dibenzyline provided five survivors out of eighteen animals and a significantly increased survival time in the non-survivors over controls. Extracorporeal circulation by itself, and in combination with Dibenzyline in precooled animals resulted in no significant differences in survival time from control animals.

Dogs in endotoxin shock have widespread vasoconstriction. Extracorporeal circulation by itself proved to be difficult to carry out as the venous return was meagre and it was often impossible to influence the blood pressure at all. When Dibenzyline was infused intravenously during the hour perfusion was carried out, a much better venous return was produced due to vasodilatation resulting from adrenergic blockade. Consequently higher perfusion rates were obtained and the blood pressure could be maintained at a mean of 90 mm. Hg. quite easily.

It is concluded that when Dibenzyline alone was infused into the shocked animals, although vasodilatation probably occurred, the myocardium was unable to recuperate sufficiently to assist the flow to the tissues. But with the help of extracorporeal circulation the flow could be maintained. Noxious products of anoxia could be removed from the tissues and oxygen supplied to them. It is suggested, on the basis of these experiments, that the combination of extracorporeal circulation and intravenous Dibenzyline is a potentially useful technique in the treatment of irreversible shock in both the experimental animal and in humans. As Dibenzyline produces adrenergic blockade for twenty-four to forty -eight hours,

it is suggested that this drug be given a trial in patients in shock, and that if no manifestation of recovery is apparent in a few hours, then the circulation should be assisted by extracorporeal means.

ADDENDUM

An analysis of 53 Gram-negative blood cultures from all the services of the University of Alberta Hospital over a three year period revealed that a total of 17 patients died, and in 11 this was due to endotoxin shock. Another 14 patients developed shock but recovered. Table V illustrates the organisms obtained. During the six year period 1956-1961, 41 patients died from endotoxin shock on the surgical services of the University of Alberta Hospital. In 38 patients the organisms and blood cultures obtained are shown in Table VI. In 3 patients no organisms were isolated. As in Table V, E.coli is again the commonest organism. In these patients the conditions complicated by septic shock are listed in Table VII. These findings substantiate those of Altemeier and Cole (2) that peritonitis is the commonest condition seen, though genito-urinary instrumentation appears to be an increasing entity.

This high mortality figure suggests that present methods of treatment are inadequate and that the search for new methods is warranted. The purpose of this addendum is to report the efficacy of Dibenzyline in the treatment of patients in septic shock. Nickerson has been one of the foremost proponents in the using of Dibenzyline in clinical shock. His rationale for its use include the following observations: 1. Most of the more reliable signs of clinical shock are also signs of vasoconstriction and of sympathetic nervous system overactivity. 2. In groups of animals subjected to a standard shocking procedure, those with evidence of greater initial sympathetic nervous system activity have the higher mortality. 3. In the presence of vasodilatation due to procedures such as high spinal cord section and an adequate blood volume, prolonged survival at very low blood pressure is possible. 4. Excessive vasoconstriction due either to exogenous sympathomimetic amines or to sympathetic nervous system overactivity, can produce or accentuate shock. 5. Elimination of reflex vasoconstriction by deafferentation of traumatised areas can increase survival at a given level of residual circulating blood volume.

Four patients have been treated with Dibenzyline, and their case summaries are recorded in Table VIII. All patients were moribund when first seen, and all had received antibiotics, vasopressor drugs, and steroids. In spite of intensive therapy all patients were continuing to deteriorate. Dibenzyline was administered intravenously in a dose of one milligram per kilogram body weight diluted in 250 ccs. of 5% dextrose and water. Vasopressors and steroids were discontinued. Two units of blood were given concurrently with the adrenergic blocking agent. This treatment was carried out over one hour during which the blood pressure was frequently recorded. As can be seen in the table, one patient did not respond. His blood pressure remained low and he was completely anuric. He died twenty-four hours later. The remaining three patients who were similarly hypotensive and anuric recovered. The one young woman made a speedy recovery in five hours, while the two older men took 24 and 48 hours respectively to recover.

This clinical resume indicates that bacteremic shock is still a considerable problem in surgical patients. Methods of treatment are by no means universally agreed upon. This is always the case when the pathophysiology of a condition is obscure. Dibenzyline would appear to be a useful adjunt to therapy in patients in extremis.

TABLE V

1959 - 1961

53 Positive Gram-negative Blood Cultures
17 Deaths

Organism	Number	Endotoxin Shock Deaths	Non Endotoxin Shock Deaths	Total Deaths
E. coli	27	4	3	7
Aer. aerogenes	12	4	1	5
Pr. vulgaris	4	2	-	2
Ps. aeruginosa	3	-	-	-
Salmonella	3	1	-	1
Paracolon	4	-	2	2
Total	53	11	6	17

TABLE VI

ORGANISMS FROM PRIMARY FOCUS AND ASSOCIATED BLOOD CULTURES IN 38 PATIENTS WITH FATAL SEPTIC SHOCK

Organisms Isolated	Number of Patients	Positive Blood Culture
E. coli	15	2
Staph. aureus	10	6
Ps. seruginosa	3	1
Cl. welchii	3	-
Pr. vulgaris	3	1
Aer. aerogenes	2	-
Heam. streptococcus	1	-
Candida albicans	1	-
Total	38	10

TABLE VII

CONDITIONS COMPLICATED BY SEPTIC SHOCK

Condition	Number	Per cent
Peritonitis	21	50.1
Genitourinary instrumentation	8	19.5
Infected burns	3	7.3
Biliary surgery	3	7.3
Wound infection	2	4.8
Septic abortion	1	11.0
Enterocolitis	1	
Cardiac surgery	1	
Gastric surgery	1	
Total	41	100

TABLE VIII

Dibenzyline in Clinical E. coli Septic Shock

Sex and Age	Diagnosis	Surgical Procedure	Shock Provoking Stimulus	Dose	Results
♀ 22	Lupus	Nil	Nil Apparent	55 mgm	B.P. Stabilised in 2 hou Urine output up 5 hours. Recovered
♂ 82	Cholecystitis	Cholecystectomy	Catheterised for retention of urine	60 mgm	B.P. maintained 3 hours Urine output normal 24 hours. Recovered
♂ 59	Carcinoma Bladder	Ileal loop	Intestinal Obstruction	70 mgm	B.P. and urine output no response. Dead 24 hours.
♂ 82	Benign Prostatic Hypertrophy	Transurethral Resection	Foley Catheter removed and reinserted	66 mgm	B.P. and urine output improved slowly over 48 hours. Recovered.

REFERENCES

1. Allen J.M., Estes J.T., and Mansberger A.R. The Use of Hypothermia in Septic Shock. The American Surgeon 26:11, 1960.

2. Altemeier W.A. and Cole W. Septic Shock. Ann. Surg. 143:600, 1956.

3. Altemeier W.A. and Cole W.R. The Nature and Treatment of Septic Shock. A.M.A. Arch. Surg. 77:498, 1958.

4. Atkins E. Pathogenesis of Fever. Physiol. Revs. 40:580, 1960.

5. Aub J.C.A Toxic Factor in Experimental Traumatic Shock. New Eng. J. Med. 23:71, 1944.

6. Baez S., Srikantia S.G. and Burack B. Dibenzyline Protection Against Shock & Preservation of Hepatic Ferritin Systems. Am. J. Physiol. 192:175, 1958.

7. Bennett I.L. and Cluff L.E. Bacterial Pyrogens. Pharm. Revs. 9:427, 1957.

8. Blair E., Buxton H.W., Cowley R.A. and Mansberger A.R. The Use of Hypothermia in Septic Shock. J.A.M.A. 178:916, 1961.

9. Borden C.W. and Hall W.H. Fatal Transfusion Reactions from Massive Bacterial Contamination of Blood. New Eng. Jour. Med. 245:760, 1951.

10. Cannon W.B. Traumatic Shock: Appleton. New York. 1923.

11. Cannon W.B. A Consideration of Possible Toxic and Nervous Factors in the Production of Traumatic Shock. Ann. Surg. 100:704, 1934.

12. Clein L.J. and Couves C.M. Septic Shock. C.M.A.J. 85:1022, 1961.

13. Clein L.J. and Couves C.M. An Evaluation of Treatment Methods in Experimental Endotoxin Shock. Surg. Forum 13:18, 1962.

14. Cockett A.T.K. and Goodwin W.E. Hypothermia as a Therapeutic Adjunct in Management of Bacteremic Shock After Urologic Surgery. Jour. Urol. 85:358, 1961.

15. Cowley R.A., Demetriades A., Mansberger A.R., Altar S., Esmond W.G., and Bessman S. Hemorrhagic Shock in Dogs Treated with Extracorporeal Circulation. A Study of Survival Time and Blood Chemistry Levels. Surg. Forum 11:110, 1960.

16. Delorme E.J. Arterial Perfusion of the Liver in Shock: An Experimental Study. Lancet i 259, 1951.

17. Dickson J.F., Hamer N.A.J. and Dow J.W. The Effect of Veno-arterial Pumping for Circulation Support. A.M.A. Arch. Surg. 80:830, 1960.

18. Einheber A. Discussion of Paper by Dr. Fine. Fed. Proc. 20:170, 1960.

19. Fine J., Frank H., Schweinberg F., Jacob S. and Gordon T. The Bacterial Factor in Traumatic Shock. Ann. N.Y. Acad. Sci. 55:429. 1952.

20. Fine J. Host Resistance to Bacterial Infection in Traumatic Shock Brit. J. Anaesth. 30:485, 1958.

21. Fine J., Frank E.D., Ravin H.A., Rutenberg S.H. and Schweinberg F.B., The Bacterial Factor in Traumatic Shock. New Eng. J. Med. 260:214, 1959.

22. Fine J., Rutenberg S. and Schweinberg F.B. The Role of the Reticulo-endothelial System in Hemorrhagic Shock. J. Exper. Med. 110:547, 1959.

23. Fine J. Endotoxins in Traumatic Shock. Fed. Proc. 20:166, 1961.

24. Frank. E.D., Frank H.A., Jacob S.W. and Fine J. Hepatic Blood Flow in Experimental Hemorrhagic Shock. Am. J. Physiol. 202:7, 1962.

25. Friedman E.W., Davidoff D. and Fine J. Hypothermia in Hemorrhagic Shock. Am. J. Physiol. 185:521, 1956.

26. Gans. H. and Krivit W. Effect of Endotoxin Shock on the Clotting Mechanism of Dogs I. Annals Surgery. 152:69, 1960.

27. Gans H. and Krivit W. Effect of Endotoxin on Clotting Mechanism II. Ann. Surg. 153:453, 1961.

28. Gans H. and Krivit W. Study of Fibrinogen and Plasminogen Concentration in Rabbits During Anaphylactic Shock. J. Lab. and Clin. Med. 58:259, 1961.

29. Gilbert R.P. Mechanisms of Hemodynamic Effects of Endotoxin. Physiol. Rev. 40:245, 1960.

30. Good R.A. and Thomas L. Studies in the Generalized Shwartzman Reaction. J. Exper. Med. 97:871, 1953.

31. Hall W. and Gold D. Shock Associated with Bacteremia: Review of 35 Cases. A.M.A. Arch. Int. Med. 96:403, 1955.

32. Hardaway R.M., Husni E.A., Geever E.F., Noyes H.E. and Burns J.W. Endotoxin Shock: A Manifestation of Intravascular Coagulation. Ann. Surg. 154:791, 1961.

33. Hardy E.G., Morris G.C., Yow E.M., Haynes B.W. and DeBakey M.E. Studies on the Role of Bacteria in Irreversible Hemorrhagic Shock in Dogs. Ann. Surg. 139:282, 1954.

34. Heymans C. and Neil E. Reflexogenic Areas of the Cardiovascular System. J. & A. Churchill Ltd., 1958.

35. Lillehei R.C. The Intestinal Factor in Irreversible Hemorrhagic Shock. Surgery, 42:1043, 1957.

36. Lillehei R.C. and MacLean L.D. The Intestinal Factor in Irreversible Endotoxin Shock. Ann. Surg. 148:513, 1958.

37. Lillehei R.C. and MacLean L.D. Physiological Approach to the Successful Treatment of Endotoxin Shock in the Experimental Animal. A.M.A. Arch. Surg. 78:464, 1959.

38. Lillehei R.C., Longerbeam J.K. and Rosenberg J.C. The Nature of Irreversible Shock: Its Relationship to Intestinal Changes. Shock: Pathogenesis and Therapy (Ciba Symposium) 1962.

39. MacLean L.D. Pathogenesis and Treatment of Bacteremic Shock. Surg. Gynec. and Obstet. 115:307. (Int. Abst.) 1962.

40. Matthes K. Myocardial Shock. Shock: Pathogenesis and Therapy. (Ciba Symposium) 1962.

41. Nickerson M. and Carter S.A. Protection Against Acute Trauma and Traumatic Shock by Vasodilators. Canad. J. Biochem. 37:1161, 1959.

42. Nickerson M. Drug Therapy of Shock. Shock: Pathogenesis and Therapy (Ciba Symposium) 1962.

43. Overton R.C. and DeBakey M.E. Experimental Observation on the Influence of Hypothermia and Autonomic Blocking Agents on Hemorrhagic Shock. Ann. Surg. 143:439, 1956.

44. Penner A. and Klein S.H. The Pathogenesis of Experimental Dysentery Intoxication. Production of the Lesions by Cerebral Circulation of the Toxin. J. Exper. Med. 96:59, 1952.

45. Peterson C.G. and Krippaehne W.W. Shock in Sepsis. Am. J. Surg. 96:158, 1958.

46. Rosen F.S. The Endotoxine of Gram-Negative Bacteria and Host Resistance. New Eng. Jour. Med. 264:919, 980, 1961.

47. Rosenberg J.C., Lillehei R.C., Longerbeam J. and Zimmermann B. Studies on Hemorrhagic and Endotoxin Shock in Relation to Vasomotor Changes and Endogenous Circulating Epinephrine, Nor-epinephrine and Serotonin. Ann. Surg. 154:611, 1961.

48. Rutenberg S.H., Schweinberg F.B. and Fine J. In Vitro Detoxification of Bacterial Endotoxin by Macrophages. J. Exper. Med. 112:801, 1960.

49. Shorr E., Zweifach B.W., Furchgott R.F. and Baez S. Hepatorenal Factors in Circulatory Homeostasis. Circulation 3:42, 1951.

50. Spink W.W. The Pathogenesis and Management of Shock Due to Infection. A.M.A. Arch. Int. Med. 106:433, 1960.

51. Thomas L. The Physiological Disturbances Produced by the Endotoxins of Gram-Negative Bacteria. J. Exper. Med. 104:865, 1956.

52. Thomas L. The Role of Epinephrine in the Reactions Produced by the Endotoxins of Gram-Negative Bacteria. J. Exper. Med. 104:865, 1956.

53. Weil M.H., Sudrann K.B. and Shubin H. Treatment of Endotoxin Shock. The Dilemma of Vasopressor and Vasodilator Therapy. California Med. 96:86, 1962.

54. Wiggers W.C. Physiology of Shock. New York Common Wealth Fund. 1950.

55. Zweifach B.W. Functional Deterioration of Terminal Vascular Bed in Irreversible Hemorrhagic Shock. Ann. N.Y. Acad. Sci. 55:370, 1952.

56. Zweifach D.W., Naglor A.L. and Thomas L. The Role of Epinephrine in the Reactions Produced by the Endotoxins of Gram-Negative Bacteria. J. Exper. Med. 104:881, 1956.

57. Zweifach B.W. Microcirculatory Derangements as a Basis for the Lethal Manifestations of Experimental Shock. Brit. J. Anaes. 30:466, 1958.

58. Zweifach B.W., Gordon H.A., Wagner M. and Reyniers J.A. Irreversible Hemorrhagic Shock in Germfree Rats. J. Exper. Med. 107:437, 1958.

59. Zweifach B.W. Aspects of Comparative Physiology of Laboratory Animals Relative to the Problem of Experimental Shock. Fed. Proc. 20:18, 1961.